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Evaluation of modifications to the neutral-detergent-fiber analysis procedure for corn and distillers grains plus solubles[1]

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ABSTRACT

Six experiments were conducted to evaluate methods for measuring NDF in corn and distillers grains plus solubles. Methods included using sodium sulfite, α-amylase (AMY), amyloglucosidase, heat and steam with an autoclave, grinding methods, and a pre–fat extraction to decrease factors that interfere with measuring NDF accurately. Using sodium sulfite and AMY resulted in decreased ($P < 0.01$) corn NDF values, but these decreases were different dependent on dry-rolled corn or high-moisture corn (interaction $P < 0.01$). Two doses of AMY (0.5 mL each) was optimum at decreasing dry-rolled corn, high-moisture corn, and steam-flaked corn NDF, but using amyloglucosidase, heat and steam with an autoclave, or both did not aid in decreasing corn NDF. Grinding corns through a 1-mm Tecator Cyclomill, compared with a Wiley mill, resulted in decreased corn NDF ($P < 0.01$). Using a pre–fat extraction method before the traditional NDF method resulted in decreased NDF values for distillers grains plus solubles ($P < 0.01$) compared with using 100 or 200 mL of NDF solution. The recommended methods for obtaining accurate NDF concentrations include using cyclo grinding and adding two 0.5 mL of AMY doses and 0.5 g of sodium sulfite for corn and pre–fat extraction for distillers grains plus solubles.

Key words: corn, distillers grains, fiber, method

INTRODUCTION

Beef, swine, and poultry in the United States are finished on high-concentrate diets containing primarily corn. However, the traditional practices for finishing beef cattle have changed over the last 20 yr with greater inclusions of ethanol by-product feeds (Klopfenstein et al., 2008). The dry-milling ethanol industry uses the starch in corn (approximately 72% of DM; Watson, 2003) to produce ethanol (Stock et al., 2000). After starch is removed, the remaining nutrients in corn are increased approximately 3-fold for distillers grains plus solubles (**DGS**; Klopfenstein et al., 2008).

The traditional NDF procedure was developed to measure the fiber content of forages and included a 1-mm grind size and 0.5-g sample weight (Van Soest and Wine, 1967). Unfortunately, measuring NDF content accurately may be difficult in high-starch (Mertens, 2002) or high-fat feeds (Van Soest, 1994), and the use of enzymes and a solvent, respectively, may help this process. Corn processing enables the starch in corn to be more available and easier to hydrolyze (Cooper et al., 2002), making NDF easier to measure. Corn hybrids can vary in endosperm and pericarp amounts (Bressani and Mertz, 1958), creating a difference in corn NDF concentration (Watson, 2003). Therefore, the objective was to determine the optimum analytical modification to the traditional NDF beaker procedure for accurately measuring NDF content in corn grain and DGS.

MATERIALS AND METHODS

Six experiments were conducted to evaluate different methodologies for determining NDF content in corn and DGS. Heat-stable α-amylase (**AMY**; 20,350 LU/mL, ANKOM Technology, Macedon, NY) and sodium sulfite

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(**SS**; crystalline, 98.6% assay, Fisher Scientific, Pittsburgh, PA) were used to digest starch and protein, respectively. All analyses were conducted at the University of Nebraska–Lincoln ruminant nutrition laboratory. A laboratory-corrected DM analysis was conducted for all samples by weighing a separate 0.5 g of sample (in duplicate; not used for NDF analysis) into a preweighed and predried aluminum pan and drying in a 105°C oven for 16 h, followed by weighing the dried sample plus aluminum pan. The NDF procedures used boiling (105°C) neutral detergent solution obtained from Midland Scientific Inc. (Davenport, IA). Following the NDF digestion process, the NDF residue was filtered on predried and preweighed Whatman grade 541 filters (12.5-cm diameter, Fisher Scientific), dried for 16 h in a 105°C oven, followed by weighing the dried filter and residue. All analytical treatments were conducted in triplicate for each sample tested within each experiment.

Exp. 1

To evaluate corn NDF content, a sample of dry-rolled corn (**DRC**) and of high-moisture corn (**HMC**) were compared using AMY and SS. One sample of each DRC and HMC were obtained on November 15, 2007, from the University of Nebraska–Lincoln Agricultural Research and Development Center research feedlot near Mead, Nebraska. Samples were dried at 60°C for 48 h and ground through a 1-mm screen (Wiley Mill; Thomas Scientific, Swedesboro, NJ). Samples were weighed (0.5 g) into tall-form, 600-mL glass beakers, and 100 mL of NDF solution (Midland Scientific Inc.) was added. Following 1 h of reflux, residue was then filtered. The 3 treatments included 1) adding 1 dose of 0.5 mL of AMY at reflux initiation with no SS; 2) adding 1 dose of 0.5 mL of AMY at reflux initiation plus weighing 0.5 g of SS into the beakers; and 3) adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation, plus adding 0.5 g of SS into the beakers.

Exp. 2

Number of AMY doses was evaluated as a means to hydrolyze starch in corn samples to accurately measure NDF content. Corns from Exp. 1 were weighed into beakers, refluxed, and filtered similarly to Exp. 1. The 3 treatments included 1) adding 1 dose of 0.5 mL of AMY at reflux initiation; 2) adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation; and 3) adding 3 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 30 and 50 min after reflux initiation. All treatments included adding 0.5 g of SS into the beakers with the corn.

Exp. 3

To eliminate any starch or fiber differences related to corn hybrids, corns of the same hybrid with different processing methods and AMY enzyme treatment were evaluated for NDF content. Samples of the same corn hybrid (Syngenta, Wilmington, DE) were processed as DRC or HMC and obtained in June 2006 from the University of Nebraska–Lincoln research feedlot. A steam-flaked corn (**SFC**) sample, not of the same hybrid but used for comparison, was also obtained from the research feedlot at the same time. Corns were dried, ground, and weighed into beakers, refluxed, and filtered similar to Exp. 1 and 2. Four enzyme treatments included 1) adding 1 dose of 0.5 mL of AMY at reflux initiation; 2) adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation; 3) adding 4 doses of 0.5 mL of AMY, 1 dose each at reflux initiation and 20, 35, and 50 min after reflux initiation; and 4) adding 2 doses of 1.0 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation. All treatments included adding 0.5 g of SS into the beakers with the corn.

Exp. 4

Combinations of AMY and amyloglucosidase (**GLU**) enzymes and treating the samples with heat and steam using an autoclave were evaluated in an attempt to hydrolyze more starch than in Exp. 1 and 2 and accurately measure NDF content. The corn samples from Exp. 3 were prepared, weighed, refluxed, and filtered similarly to previous experiments for Exp. 4, which contained 4 treatments. Treatment 1 included adding 0.5 mL of AMY at reflux initiation, refluxing for 30 min, cooling beakers until solution reached 50°C, adding 0.5 mL of GLU, allowing samples to sit for 10 min then refluxing again for 30 min, and adding 0.5 mL of AMY 10 min before removing beakers from the end of the reflux step (**AMY-GLU-AMY-0.5**). Treatment 2 included adding 1 mL of AMY at reflux initiation and 50 min after reflux initiation with a 1-h reflux duration. Beakers were then removed, and when the solution reached 50°C, 1 mL of GLU was added and allowed to sit for 10 min before filtering (**AMY-AMY-GLU-1**). Treatment 3 included adding steam and heat at 121°C for 30 min to corn samples residing in NDF beakers in an autoclave (**AUT**) then refluxing with 1 mL of AMY at reflux initiation and reflux for 1 h (**AUT-AMY-1**). Treatment 4 included using the same AUT process, refluxing for 1 h, adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation, letting the solution cool to 50°C, and adding 0.5 mL of GLU before filtering (**AUT-AMY-AMY-GLU-0.5**). All treatments included adding 0.5 g of SS into the beakers with the corn.

Exp. 5

Grinding methods for corns were evaluated to determine NDF content. Samples of DRC included the same samples from Exp. 1 and 2, the same samples from Exp. 3 and 4, and 2 corn samples obtained from Poet Nutrition (Sioux Falls, SD) on Sep-

tember 12, 2006. Corn samples were dried, weighed, refluxed, and filtered similarly to all previous experiments. The 2 treatments included 1) grinding samples through a 1-mm-screen Wiley mill or 2) grinding samples through a 1-mm Tecator Cyclotec sample mill (American Instrument Exchange, Haverhill, MA). Both treatments included adding 0.5 g of SS into the beakers with the corn and using 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation.

Exp. 6

High-fat (>5% fat) dried DGS (**DDGS**) samples were used to evaluate different amounts of NDF solution and a pre-NDF reflux, fat extraction method for determining NDF content. Five DDGS samples (POET Nutrition) with differing amounts of solubles added to the grains portion were obtained from the University of Nebraska–Lincoln research feedlot. These samples had 0, 33, 67, 100, and 110% the normal incorporation of solubles to distillers grains (Corrigan et al., 2009). Three analytical treatments were evaluated for these samples: 1) the traditional Van Soest and Wine (1967) method explained in Exp. 1 plus an acetone rinse at filtering, 2) the same as method 1 but with 200 mL of NDF solution, and 3) using a bi-phasic fat extraction method using a 1:1 ratio of hexanes and diethyl ether described by Bremer et al. (2010a) then rinsing the nonlipid residue into a 600-mL, tall-form beaker with 100 mL of NDF solution and applying an acetone rinse at filtering. The fat was measured for these samples. All treatments included grinding the samples through a Wiley mill (1-mm screen), adding 0.5 g of SS into the beakers, and adding 0.5 mL of AMY at reflux initiation.

Statistical Analysis

Data were analyzed using the Proc Mixed procedure of SAS (Version 9.2, SAS Institute Inc., Cary, NC) for each experiment. Sample type and analytical treatment were considered fixed effects, and the individual observation within method was considered an experimental unit. Interactions between sample type and analytical treatment were tested for significance. When no significant interactions were observed ($P > 0.05$), main effects of sample type and analytical treatment are presented. When significant interactions were observed ($P \leq 0.05$), simple effects are presented.

RESULTS AND DISCUSSION

Exp. 1

An interaction resulted for NDF concentration between corn sample and analytical treatment ($P < 0.01$; Table 1). This suggests the use of SS and AMY was not consistent in extracting nonfibrous materials from DRC and HMC. Using SS to extract protein that was complexed with NDF resulted in decreased ($P < 0.01$) NDF values when AMY level remained constant. This agrees with Van Soest (1994), who stated that protein can be complexed with lignin in numerous feeds and the protein can be dissolved by using SS, resulting in lower NDF values. Therefore, 0.5 g of SS was used in all subsequent NDF procedures that were conducted.

When SS was included, there continued to be an interaction ($P < 0.01$) between corn sample and number of AMY doses. With HMC, increasing the number of AMY doses from 1 to 2 decreased ($P < 0.01$) the NDF content from 17.20 to 8.85% DM. This agreed with the NRC (1996), which stated the NDF content in corn is 9 to 10% of DM, dependent on bushel weight. Mertens (2002) also stated 8.0 to 8.1% NDF values for treating corn samples with AMY. However, increasing the number of AMY doses for the DRC sample resulted in increased ($P < 0.01$) NDF content from 12.30 to 14.27% DM. Neither of these NDF results for DRC appeared to be acceptable values for corn NDF. When filtering these DRC samples, the filters appeared to retain some visual granular material that appeared to be nonfiber material, perhaps the germ or endosperm. Although there was only one sample of DRC and HMC with limited replication, further analysis of these samples follows in other experiments for increased statistical significance.

Exp. 2

An interaction resulted between analytical technique (number of AMY doses) and corn sample ($P < 0.01$; Table 1). Increasing the number of AMY doses from 1 to 2 decreased ($P < 0.01$) the NDF content for both DRC (26.81 vs. 12.63%, respectively) and HMC (16.45 vs. 10.16%, respectively). This decrease in NDF values was presumably due to increased starch removal. Mertens (2002) suggested that starch in feeds can be difficult to hydrolyze by only using NDF solution, and AMY can be used in the NDF procedure to facilitate this process. However, he indicated that considerable variability can occur depending on the laboratory or the type of AMY used. No differences in NDF content resulted between dosing AMY 2 or 3 times within each corn type ($P \geq 0.50$). These results suggested that more corn starch was hydrolyzed when AMY doses increased from 1 to 2 with smaller changes from 2 to 3 AMY doses.

The NDF values for HMC, when dosing 2 (10.16%) or 3 (10.05%) times with AMY, were similar to those stated in the NRC (1996). Within each analytical method in this experiment, the NDF values for DRC were greater than those for HMC ($P \leq 0.05$), and these DRC values continued to be greater than those reported by the NRC (1996) and Mertens (2002). When filtering these DRC samples, the filters continued to contain some granular, nonfibrous material. The results from Exp. 1 and 2 indicate that starch removal is incomplete (greater NDF values) if adequate starch hydrolyzing steps are not taken.

Table 1. NDF[1] content of dry-rolled corn (DRC) or high-moisture corn (HMC) samples obtained in November 2007 when treated with different doses of α-amylase (AMY) and sodium sulfite (SS) in Exp. 1 and additional doses of AMY in Exp. 2

	Treatment for Exp. 1[2]							
	1 AMY NO SS		1 AMY		2 AMY			
Item	DRC–Nov. 2007	HMC–Nov. 2007	DRC–Nov. 2007	HMC–Nov. 2007	DRC–Nov. 2007	HMC–Nov. 2007	SEM[3]	Interaction[4]
NDF	33.58[f]	21.81[e]	12.30[b]	17.20[d]	14.27[c]	8.85[a]	0.59	<0.01
	Treatment for Exp. 2[5]							
	1 AMY		2 AMY		3 AMY			
	DRC–Nov. 2007	HMC–Nov. 2007	DRC–Nov. 2007	HMC–Nov. 2007	DRC–Nov. 2007	HMC–Nov. 2007	SEM[3]	Interaction[4]
NDF	26.81[d]	16.45[c]	12.63[ab]	10.16[a]	13.58[b]	10.05[a]	0.97	<0.01

[a–f]Means in the same row without a common superscript differ ($P < 0.05$).

[1]Values expressed on a % of DM basis.

[2]Where 1 AMY NO SS = adding 1 dose of 0.5 mL of AMY at reflux initiation with no SS; 1 AMY = adding 1 dose of 0.5 mL of AMY at reflux initiation and weighing 0.5 g of SS into beakers with corn before reflux process; and 2 AMY = adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation and adding 0.5 g of SS into beakers with corn before reflux process.

[3]Each treatment mean represents 3 replicates (n).

[4]Where Interaction = *P*-value for *F*-test of interaction between corn sample and analytical treatment.

[5]Where 1 AMY = adding 1 dose of 0.5 mL of AMY at reflux initiation; 2 AMY = adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation; 3 AMY = adding 3 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose each at 30 and 50 min after reflux initiation. All 3 treatments included adding 0.5 g of SS in beakers with corn.

Exp. 3

No interaction ($P = 0.93$) was observed between analytical treatment and corn type (Table 2). A statistical effect was not observed among corn samples ($P = 0.47$), likely because of inherent analytical error and the relatively small differences among samples. The amount of endosperm and pericarp in corn changes for different corn hybrids (Bressani and Mertz, 1958) but should remain the same for the same corn hybrid. This is important because the endosperm contains about 85% starch and almost no NDF, but the pericarp is about 90% NDF (Watson, 2003).

Increasing the dose of AMY from 1 to 2 at 0.5 mL decreased ($P < 0.01$) the corn NDF content measured. The NDF values were 21.82 and 13.08% for 1 and 2 AMY doses, respectively. However, increasing the doses of AMY from 2 to 4 did not further decrease NDF ($P = 0.53$). The hypothesis was that increasing the dosing amount of AMY from 0.5 to 1.0 mL would hydrolyze more starch and lower the NDF content. However, no difference ($P \geq 0.63$) was observed for dosing AMY twice at 1 mL compared with dosing AMY 2 or 4 times at 0.5 mL each. In this experiment, increasing the AMY dose from 1 to 2 appeared to hydrolyze more starch and result in a more accurate corn NDF value, but increasing AMY beyond 2 doses at 0.5 mL each did not appear to hydrolyze more starch. Regardless of AMY dosage and concentration, visual granular material continued to be observed remaining on the filters that did not appear fibrous. Therefore, this suggests the NDF results from this experiment were not indicative of true NDF content.

Exp. 4

An interaction resulted between corn sample and analytical treatment of enzymes in combination with AUT ($P < 0.01$; Table 3). Using different combinations of AMY and GLU enzymes in treatments AMY-GLU-AMY-0.5 and AMY-AMY-GLU-1 resulted in mixed results for the corn samples. Neither enzyme combination treatment appeared to be superior at increasing starch hydrolysis and reducing NDF values. Using an enzyme treatment alone decreased NDF values for the DRC and SFC samples ($P < 0.01$) compared with incorporating AUT. The prediction was that using GLU in combination with AMY would hydrolyze the difficult glucose bonds in the nonreducing ends of starch to result in acceptable NDF values. However, the NDF values observed in this experiment were not as

Table 2. NDF[1] content of the same corn hybrid processed as dry-rolled corn (DRC) or high-moisture corn (HMC) and a steam-flaked corn (SFC) sample obtained in June 2006 when treated with different doses of α-amylase (AMY) in Exp. 3[2]

	Corn type[3]				Analytical treatment[4]				
Item	DRC hyb	HMC hyb	SFC	*P*-value[5]	1 AMY-0.5 mL	2 AMY-0.5 mL	4 AMY-0.5 mL	2 AMY-1 mL	*P*-value[6]
NDF	15.86	14.53	14.59	0.47	21.82[b]	13.08[a]	12.20[a]	12.88[a]	<0.01

[1]Values expressed on a % of DM basis.

[2]*F*-test of interaction between corn sample and analytical treatment *P*-value = 0.93.

[3]Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, HMC hyb = Golden Harvest H-8562 hybrid processed as HMC, SFC = corn processed as SFC.

[4]Where 1 AMY-0.5 mL = adding 1 dose of 0.5 mL of AMY at reflux initiation; 2 AMY-0.5 mL = adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation; 4 AMY-0.5 mL = adding 4 doses of 0.5 mL of AMY, 1 dose each at reflux initiation and 20, 35, and 50 min after reflux initiation; and 2 AMY-1 mL = adding 2 doses of 1 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation. All 4 treatments included adding 0.5 g of sodium sulfite in beakers with corn.

[5]*P*-value for *F*-test differences among corn samples. SEM = 0.85; each treatment mean represents 16 replicates (n).

[6]*P*-value for *F*-test differences among analytical treatments. SEM = 0.98; each treatment mean represents 12 replicates (n).

acceptable (not as close to 9 to 10%) as the values in Exp. 3 in which 2 doses of AMY were used in a continuous refluxing process.

The hypothesis was that using AUT would help hydrolyze starch and make it more available for enzyme utilization, similar to how SFC is processed with steam and pressure. Using AUT lowered the NDF content for HMC compared with the DRC and SFC samples ($P < 0.01$), but the NDF values for HMC remained above (15.28 and 12.13% for AUT-AMY-1 and AUT-AMY-AMY-GLU-0.5, respectively) acceptability. However, AUT was not successful at hydrolyzing starch in the DRC and SFC samples; NDF values remained above 30 and 15% for the AUT-AMY-1 and AUT-AMY-AMY-GLU-0.5 treatments, respectively. Therefore, 2 doses of AMY were continued to be used to hydrolyze starch in the subsequent corn experiments.

Exp. 5

An interaction resulted between DRC samples and grind type for NDF content ($P < 0.01$; Table 4). This interaction was because the marginal decrease was different for grinding through the Cyclomill compared with the Wiley mill among samples. The NDF values observed for the 4 DRC samples ground through the Wiley mill (range = 13.77 to 17.66% DM) were considered above acceptability. Granular material residing on the filters continued to be visually observed for Wiley grinding that did not appear to be fiber similar to previous experiments.

When the DRC samples were ground through the Cyclomill, not only did the samples result in lower NDF values ($P < 0.01$), but no visual granular material remained on the filters. Three of the 4 samples resulted in NDF values of 9.74 to 10.60% DM, which we considered acceptable for corn. We realize the second Poet Nutrition DRC sample resulted in an NDF value of 7.56% DM, but this sample replicated very well (SD = 0.1). This difference may be due to corn hybrid differences as stated previously or is realistic based on other observations for corn NDF values of 8.0 to 8.1% (Mertens, 2002).

These results indicated that if corn processing or enzyme treatment are different for measuring corn NDF content, then the resulting values may vary substantially. Dairy One Forage Analysis Laboratory (2010) summarized 263 corn samples from 2000 to 2010 and reported an average NDF value of 18.90% DM, with a normal range of 12.99 to 24.81% using the ANKOM method (ANKOM Technology, Macedon, NY) and dosing with AMY and adding SS into the machine. These numbers remained above what the NRC (1996) stated, which used the ANKOM methods, and what was observed currently. The NDF analyses obtained for corn samples using an ANKOM filter bag machine do not appear to be consistent. If unwanted starch remains in filter bags, then NDF values would be greater than expected.

In the traditional NDF beaker system, dosing once with 0.5 mL of AMY was not sufficient at hydrolyzing starch, and 2 AMY doses were needed with 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation to allow time for the enzyme to work at its full potential. Finally, this analytical procedure is not accurate unless the corn samples have been ground fine enough (i.e., through a 1-mm-screen Cyclomill) to degrade the corn starch. These combined techniques result in corn NDF values that are comparable to the NRC (1996).

Table 3. NDF[1] content of the same corn hybrid processed as dry-rolled corn (DRC) or high-moisture corn (HMC) and a steam-flaked corn (SFC) sample obtained in June 2006 when treated with different doses of α-amylase (AMY), amyloglucosidase (GLU), and pressurizing with steam and heat in an autoclave (AUT) in Exp. 4[2,3]

	AMY-GLU-AMY-0.5			AMY-AMY-GLU-1			AUT-AMY-1			AUT-AMY-AMY-GLU-0.5			
Item	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	DRC hyb	HMC hyb	SFC	Interaction[4]
NDF	15.16^{de}	14.02^{cd}	12.02^{ab}	13.81^{bcd}	12.36^{abc}	11.26^{a}	32.87^{g}	15.28^{de}	35.50^{h}	20.53^{f}	12.13^{ab}	16.35^{e}	<0.01

$^{a–h}$Means in the same row without a common superscript differ ($P < 0.05$).

[1]Values expressed on a % of DM basis.

[2]Analytical treatment: AMY-GLU-AMY-0.5 = adding 1 dose of 0.5 mL of AMY at reflux initiation, refluxing for 30 min, setting aside beakers until solution reached 50°C, adding 0.5 mL of GLU, setting aside for 10 min then refluxing again for 30 min, and adding 0.5 mL of AMY 10 min before the end of the reflux process; AMY-AMY-GLU-1 = adding 2 doses of 1 mL of AMY at reflux initiation and 50 min after reflux initiation, setting aside beakers until solution reached 50°C, adding 1 mL of GLU, and letting beakers sit for 10 min before filtering; AUT-AMY-1 = using an AUT at 121°C for 30 min, starting reflux process, and adding 1 mL of AMY at reflux initiation and refluxing for 1 h; AUT-AMY-AMY-GLU-0.5 = using an AUT at 121°C for 30 min, refluxing for 1 h, adding 2 doses of 0.5 mL of AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation, setting aside beakers until solution reached 50°C, adding 0.5 mL of GLU, and letting beakers sit for 10 min before filtering. All 4 treatments included adding 0.5 g of sodium sulfite in beakers with corn.

[3]Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, HMC hyb = Golden Harvest H-8562 hybrid processed as HMC, SFC = corn processed as SFC.

[4]Where Interaction = *P*-value for *F*-test of interaction between corn sample and analytical treatment. SEM = 0.64; each treatment mean represents 3 replicates (n).

Exp. 6

The NDF content for DDGS decreased ($P < 0.01$) as the ratio of solubles to distillers grains increased up to 100% of normal inclusion, regardless of analytical treatment (Table 5). This was expected as solubles contain very little NDF (2 to 8% DM; Bremer et al., 2010b). However, an interaction resulted between DDGS sample and analytical technique ($P < 0.01$), which was due to inconsistent results between the traditional and added NDF solution treatments, particularly at 100 and 110% solubles added to grains. Adding twice as much NDF solution to the procedure did not decrease NDF content for all of the samples. The hypothesis was that the additional NDF solution would be useful in solubilizing additional fat from the DDGS samples compared with the traditional procedure, but this did not occur.

As expected, fat content increased as level of solubles was added to the distillers grains ($P < 0.01$; 7.1 to 13.9% fat, DM basis). Solubles typically contain 18 to 28% fat, and Bremer et al. (2010b) observed 23.6% fat. Therefore, using a pre–fat extraction process (Bremer et al., 2010a) before the traditional NDF method to decrease interacting factors between fat and fiber in high-fat (>5% fat) samples appeared to be logical. This procedure resulted in decreased ($P < 0.01$) NDF content for each DDGS sample. A decrease of 4.5 to 5.9% units was observed when using the pre–fat extraction step before refluxing with NDF solution compared with the traditional NDF procedure. The NDF content of these DDGS with the pre-fat procedure was 26.69 to 37.29% DM and varied because of solubles inclusion.

Dairy One Forage Analysis Laboratory (2010) analyzed 4,794 DGS samples for NDF content in an ANKOM filter bag machine and determined an average NDF content of 33.85% DM with a normal range of 29.28 to 38.43%. This range can be due to varying levels of solubles added to the distillers grains or incomplete

Table 4. NDF[1] content of 4 dry-rolled corn (DRC) samples ground through a 1-mm screen in a Wiley mill (Wiley) or a Tecator Cyclomill (Cyclo) using 2 doses of α-amylase (AMY) in Exp. 5[2,3]

	DRC hyb		DRC–Nov. 2007		DRC-Poet 1		DRC-Poet 2			
Item	Wiley	Cyclo	Wiley	Cyclo	Wiley	Cyclo	Wiley	Cyclo	SEM[4]	Interaction[5]
NDF	13.77[c]	10.60[b]	16.70[d]	10.38[b]	17.66[d]	9.74[b]	14.85[c]	7.56[a]	0.55	<0.01

[a–d]Means in the same row without a common superscript differ ($P < 0.05$).

[1]Values expressed on a % of DM basis.

[2]Where DRC hyb = Golden Harvest H-8562 hybrid processed as DRC, DRC–Nov. 2007 = DRC sample obtained in November 2007, DRC-Poet 1 = corn sample 1 obtained from Poet Nutrition (Sioux Falls, SD) and processed as DRC, DRC-Poet 2 = corn sample 2 obtained from Poet Nutrition and processed as DRC.

[3]Where Wiley = ground sample through a 1-mm screen Wiley Mill, Cyclo = ground sample through a 1-mm screen Tecator Cyclomill. All treatments included dosing twice with 0.5 mL AMY, 1 dose at reflux initiation and 1 dose at 50 min after reflux initiation, and adding 0.5 g sodium sulfite in beakers with corn.

[4]Each treatment mean represents 3 replicates (n).

[5]Where Interaction = *P*-value for *F*-test of interaction between corn sample and analytical treatment.

Table 5. NDF and fat[1] content of dried distillers grains plus solubles (DDGS) with different ratios of grains to solubles when using the traditional NDF procedure with 100 mL of NDF solution, 200 mL of NDF solution, or conducting a pre–fat extraction followed by the traditional NDF procedure with 100 mL of NDF solution[2]

	Analytical treatment[4]			
Sample[3]	100 mL NDF	200 mL NDF	Pre–fat NDF (100 mL)	Fat
0DDGS	43.40[k]	41.61[j]	37.29[i]	7.1
33DDGS	38.07[i]	37.93[i]	32.72[fg]	9.2
67DDGS	33.58[g]	34.82[h]	28.96[c]	10.8
100DDGS	31.32[de]	32.61[fg]	27.51[b]	12.8
110DDGS	31.79[ef]	30.69[d]	25.69[a]	13.9

[a–k]Means with different superscripts differ ($P < 0.05$).

[1]Values expressed on a % of DM basis.

[2]Interaction with an *F*-test resulted in a *P*-value of <0.01 between DDGS sample and analytical treatment. SEM = 0.59; each treatment mean represents 3 replicates (n).

[3]Where 0DDGS = 0% of traditional amount of solubles added to distillers grains; 33DDGS = 33% of traditional amount of solubles added to distillers grains; 67DDGS = 67% of traditional amount of solubles added to distillers grains; 100DDGS = 100% of traditional amount of solubles added to distillers grains; 110DDGS = 110% of traditional amount of solubles added to distillers grains.

[4]Where 100 mL NDF = using the traditional Van Soest and Wine (1967) procedure with 100 mL of NDF solution; 200 mL NDF = using the traditional method with 200 mL of NDF solution; Pre–fat NDF (100 mL) = conducting a pre–fat extraction with 1:1 hexanes and diethyl ether on the samples and rinsing the residue into beakers with 100 mL of NDF solution. Fat content was measured using this method. All treatments included weighing 0.5 g of sodium sulfite in beakers with corn, dosing with 0.5 mL of α-amylase at reflux initiation, and rinsing filters with acetone.

removal of fat from the filter bags for NDF analysis. Therefore, using the traditional beaker method coupled with the pre–fat extraction process is appropriate to measure NDF in high-fat feeds because NDF solution alone cannot solubilize large quantities of fat in feeds.

IMPLICATIONS

Starch and fat can interfere with fiber analysis in corn and DGS, respectively, resulting in inaccurate NDF analytical results. Using 2 doses of AMY during the NDF refluxing process is required to hydrolyze starch in corn. However, corns should also be preground through the Cyclomill to physically expose starch for sufficient starch hydrolysis and to obtain accurate NDF values. A bi-phasic solvent, fat-extraction process should be conducted on DGS samples before the traditional beaker NDF procedure for solubilizing fat and measuring accurate NDF.

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